PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 030832wo CS-gn	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/08447	International filing date *(day/month/year)* 30.07.2003	Priority date *(day/month/year)* 31.07.2002
International Patent Classification (IPC) or both national classification and IPC C07H21/00		
Applicant GIRINDUS AG		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.02.2004	04.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Fitz, W Telephone No. +31 70 340-4359

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/08447

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-60 as originally filed

Claims, Numbers

1-14 received on 14.10.2004 with letter of 13.10.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	13
	No:	Claims	1-12,14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 01 51532 A (UNIV MICHIGAN) 19 July 2001 (2001-07-19)
D2: US-B1-6 306 599 (PERBOST MICHEL G M) 23 October 2001 (2001-10-23)
D3: US-A-5 808 042 (TAN WEITIAN ET AL) 15 September 1998 (1998-09-15) cited in the application

1.) The subject-matter of claims 1-12 and 14 is new in the sense of Article 33(2) PCT, because the available prior art documents do not disclose a method for the preparation of an oligonucleotide wherein both coupling and deprotection are effected with solid supported reagents.
The subject-matter of claim 13 is new because the available prior art documents do not disclose the use of a solid supported amine and a tetrathionate having the formula S_4O_6 or a cyanoethylthiosulfate ($NC-CH_2-CH_2-S-SO_3-$) for the sulfurization of oligonucleotides.

2.) The subject-matter of claims 1-12 and 14 does not involve an inventive step in the sense of Article 33(3) PCT. The reasons for this are the following:
Documents D1 and D2, each of which can be considered to represent the most relevant state of the art, disclose the use of solid supported activators for the synthesis of oligonucleotides, from which the subject-matter of claims 1,3 and 14 differs in that not only the coupling step but also the deprotection step is effected by a solid supported reagent.
In view of the teachings of D1 or D2, the problem underlying the application is considered as the provision of a further method for preparing an oligonucleotide.
The solution proposed in claims 1, 3 and 14, i.e. using a solid supported activator in the coupling step and performing the deprotection step by treatment with a solid supported agent or with a removal agent followed by addition of a solid supported scavenger, cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.
Document D3 discloses a method for the detritylation of chemically synthesized oligonucleotides wherein a solid supported scavenger is used.
The skilled person, being knowledgeable in the field of oligonucleotide synthesis, would therefore regard it as a normal design procedure to incorporate the detritylation method of D3 into the method of D1 and D2. He would expect with the clear anticipation of

success that the detritylation method of D3 could be incorporated into the synthesis cycles of D1 or D2. He would thus arrive at claims 1, 3 and 14 in an obvious manner. Dependent claims 2,4-12 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.
The subject-matter of claim 13 is considered as involving an inventive step, because the available prior art documents do not suggest the use of a solid supported amine and a tetrathionate having the formula S_4O_6 or a cyanoethylthiosulfate ($NC-CH_2-CH_2-S-SO_3-$) for the sulfurization of oligonucleotides.

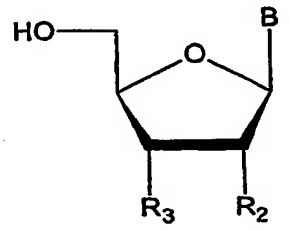
3.) Claims 1-14 are considered as industrially applicable because the prepared oligonucleotides are useful for pharmaceutical purposes.

4.) The terms "solid supported activator" of step b), as well as "solid supported agent", "removal agent" and "solid supported scavenger" of step d) of claims 1, 3 and 14 are vague and leave the reader in doubt as to their exact meaning.
Furthermore these functional terms do not enable the skilled person to determine, without undue burden, which technical features are necessary to perform the stated functions - Art. 5 and 6 PCT.

Claims

1. A method for preparing an oligonucleotide comprising the steps of

a) providing a 3′-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2′-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′-O2′methylen linkage

R_3 is OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

b) reacting said compound with a nucleotide derivative having a 5′-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

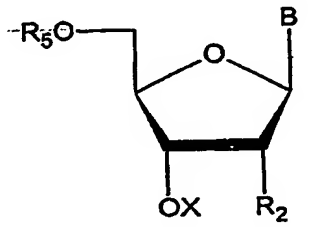
c1) capping preferably by reacting with a solid supported capping agent

c2) oxidizing preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 5′-protection group by treatment with a solid supported agent or removing the 5′-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.

2. The method of claim 1, wherein the nucleotide derivative having a 5'-proctection group of step b) has the following formula:



wherein

X is a P(III)-function

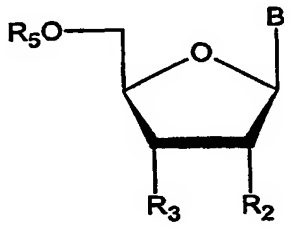
B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide.

3. A method for preparing an oligonucleotide comprising the steps of

a) providing a 5'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 is OH, NH_2

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide

b) reacting said compound with a nucleotide derivative having a 3'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

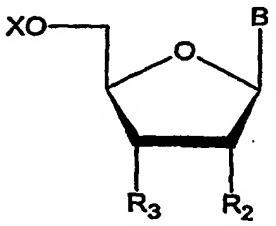
c1) capping, preferably by reacting with a solid supported capping agent

c2) oxidizing, preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3'-protection group by treatment with a solid supported agent or removing the 3'-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.

4. The method of claim 3, wherein the nucleotide derivative having a 3'-proctection group has the following formula:



wherein

X is a P(III)-function

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 = OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

R°_3 is a hydroxyl protecting group, a 3'-protected nucleotide or a 3'-protected oligonucleotide.

5. The method of any one of claims 1 to 4, wherein the nucleotide derivative of step b) is a phosphoramidite or a H-phosphonate.

6. The method of any one of steps 1 to 5, wherein the solid supported activator of step b) is selected from the group consisting of a solid support bearing a pyridinium salt, a cation exchange solid support with an optionally substituted pyridinium, a cation exchange solid support with an optionally substituted imidazolium salt, a solid support bearing an optionally substituted azole (imidazol, triazole, tetrazole), a salt of a weak base anion exchange resin with a strong acid, a weak cation exchange resin (carboxylic) in its protonated form, a solid support bearing an optionally substituted phenol, a solid support bearing a carboxylic acid chloride/bromide, a sulfonic acid chloride/bromide, a chloroformate, a bromoformate, a chlorosulfite, a bromosulfite, a phosphorochloridate, a phosphorbromidate and a solid support bound carbodiimide.

7. The method of any one of claims 1 to 6, wherein the solid supported oxidizing reagent is selected from the group consisting of solid supported periodates, permanganates, osmium tetroxides, dichromates, hydroperoxides, substituted alkylamine oxides, percarboxylic acid and persulfonic acid.

8. The method of any one of claims 1 to 7, wherein the oxidizing is a sulfurization.

9. The method of claim 8, wherein the solid supported oxidizing reagent is selected from the group consisting of a solid supported tetrathionate, a solid supported alkyl or aryl sulfonyl disulfide, a solid supported optionally substituted dibenzoyl tetrasulfide, a solid supported bis(akyloxythio-

carbonyl)tetrasulfide, a solid supported optionally substituted phenylacetyl disulfide, a solid supported N-[(alkyl or aryl)sulfanyl] alkyl or aryl substituted succinimide and a solid supported (2-pyridinyldithio) alkyl or aryl.

10. The method of any one of claims 1 to 9, wherein the solid supported capping agent is a solid supported activated acid, preferably a carboxylic acid chloride, carboxylic acid bromide, azolide, substituted azolide, anhydride or chloroformate or phosphorochloridate, or a solid supported phosphoramidite, or a solid supported H-phosphonate monoester.

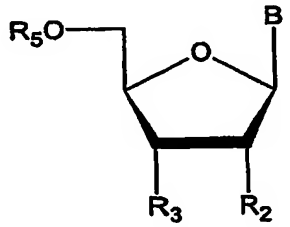
11. The method of any one of claims 1 to 10, wherein the 5′-protection is a dimethoxytrityl group (DMTr) or a monomethoxytrityl group (MMTr) and the solid supported agent of step d) is an cationic ion exchanger resin in the H^+ form or solid supported ceric ammonium nitrate.

12. The method of any one of claims 1 to 11, wherein the 3′-protection is a silyl group and the solid supported agent of step d) is an anionic ion exchanger resin in the F-form or the 3′-proctection is levulinic acid and the solid supported agent of step d) is a solid supported hydrazine or a solid supported hydrazinium.

13. Use of a solid supported sulfurization agent consisting of solid supported amine and a tetrathionate having the formula S_4O_6 or a cyanoethylthiosulfate ($NC\text{-}CH_2\text{-}CH_2\text{-}S\text{-}SO_3^-$) for sulfurization of oligonucleotides.

14. A method for preparing an oligonucleotide comprising the steps of

a) providing a compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2′-hydroxyl group, F, a protected amino group, an O-

alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′-O2′methylen linkage

and

R_3 is OR′$_3$, NHR″$_3$, NR″$_3$R‴$_3$,

a protected nucleotide or a protected oligonucleotide and R_5 is a P(III) function

R′$_3$ is a hydroxyl protecting group,

R″$_3$, R‴$_3$ are independently an amine protecting group,

or

R_5 is a hydroxyl protecting group, a protected nucleotide or a protected oligonucleotide and R_3 is a P(III) function

b) reacting said compound with a nucleotide derivative having a 3′ or 5′-free OH-group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

c1) capping by reacting with a solid supported capping agent

c2) oxidizing by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3′ or 5′-protection group by treatment with a solid supported agent or removing the 5′-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.